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Abstract

Bacteriophages are increasingly used as tracers for quantitative analysis in both hydrology and hydrogeology. The biological particles are neither toxic nor pathogenic for other living organisms as they penetrate only a specific bacterial host. They have many advantages over classical fluorescent tracers and offer the additional possibility of multi-point injection for tracer tests. Several years of research make them suitable for quantitative transport analysis and flow boundary delineation in both surface and ground waters, including karst, fractured and porous media aquifers.

This article presents the effective application of bacteriophages based on their use in differing Swiss hydrological environments and compares their behaviour to conventional coloured dye or salt-type tracers. In surface water and karst aquifers, bacteriophages travel at about the same speed as the typically referenced fluorescent tracers (uranine, sulphurhodamine G extra). In aquifers of interstitial porosity, however, they appear to migrate more rapidly than fluorescent tracers, albeit with a significant reduction in their numbers within the porous media. This faster travel time implies that a modified rationale is needed for defining some ground water protection area boundaries. Further developments of other bacteriophages and their documentation as tracer methods should result in an accurate and efficient tracer tool that will be a proven alternative to conventional fluorescent dyes.

Introduction

Artificial tracers have been used for some time to estimate and quantify the nature, direction and rate of flow. However, despite the large number of available chemical tracers (Käss, 1992) surface and ground water specialists still continue to search for ideal substances to use as tracers in varying hydrological environments. Today, relatively few substances qualify as tracers. To qualify, a chemical substance or suspended particle must behave with relatively stringent properties. It must be stable, mix well with water and have a density close to it. This tracer has to be detected and quantified in minute concentrations using simple procedures that are of low cost. Furthermore, it should not be toxic or have environmental pollution potential, interact with materials naturally found in the media, or leave any residual background levels in the aquifer. These constraints are considerable and only a limited number of substances come close to meeting the criteria for the 'ideal' tracer. It is particularly limiting when there is a strong rationale or need to perform multi-point experiments with different tracers injected at the same time at different locations.

Successful tracer tests conducted in the Swiss Karst Jura (Aragno and Müller, 1982) initially encouraged the development of a systematic experimental approach using microbiological tracers, notably, bacteriophages. However, several years of research were necessary to provide hydrologists with an effective method of tracer testing in both surface and ground water flow systems. This new class of tracers allows flow systems to be followed as well as providing key information for the study of the movement and migration patterns of colloidal particles. Thus, bacteriophages have opened up a number of truly multidisciplinary research opportunities.

Biological tracers

Biological tracers differ from chemical tracer solutions which have been more commonly employed in the past. They are present in water as suspended particles of microscopic size (colloids), rather than as dissolved chemicals. The particular biological materials used as tracers (spores, bacteria, viruses) are organisms, living or dead, that range in size from tens to several hundreds of nanometers (nm). The critical condition that must be documented before they are allowed to be used as tracers, is that they have no adverse impact on humans or on the environment. Keswick *et al.*, 1982, and Wimpenny *et al.*, 1972 specified that they should:

1. Be neither pathogenic nor toxic,
2. Be not naturally present in ground water, but if they are, able to be distinguished rapidly from the indigenous organism (e.g. using a genetic marker),
3. Not affect the flow rate,
4. Be stable under the environmental conditions to which they will be subjected during testing,
5. Move with the water flow, being neither filtered from, nor adsorbed upon the aquifer materials,
6. Be quantified using an enumeration/counting technique that is both rapid and inexpensive,
7. Neither interact with nor modify other micro-organisms,
8. If needed, be able to be combined simultaneously with several different tracers without sample or interpretation interference in the analyses.

BACTERIOPHAGES AS BIOLOGICAL TRACERS FOR HYDROLOGY:

Following a discussion by Wimpenny (1972) on bacteriophages, the Microbiology Laboratory and the Centre of Hydrogeology at the University of Neuchâtel cooperated in research into the use of bacteriophages as biological tracers in hydrogeology (Carvalho Dill, 1993; Rossi, 1994; Dörfliger, 1997). An efficient means of analyzing phage tracer test data quantitatively was developed; this that was pertinent not only in fractured and karst terrain, but now also, following tracer testing results from the Wilerwald site in Canton Bern, in granular porous media. Earlier results (Carvalho Dill, 1993; Rossi *et al.*, 1994) had shown fast tracer transit times at the site and had pointed out the need for more frequent sampling to quantify the subsurface system's response.

Bacteriophage, or simply, phage, signifies 'that which feeds on bacteria'. This term represents a virus which, uniquely, invades specific bacterial cells and has no effect on human, animal or vegetal cells. Like all viruses, bacteriophages are incapable of multiplying in an independent manner. Viruses have to be integrated physically within a specific host cell. When a phage enters its specific bacterial host, it takes control of it and starts the production of new phages which will be released at the end of the infection cycle.

Numerous types of phages appear in the environment and in widely diverse locations (Table 1). Börsheim (1993) showed that concentrations of viral particles in both fresh and sea water varied from 10^3 to 10^7 per ml. They can even reach levels of concentration as high as 4.6×10^8 viral particles per ml, the highest recorded level ever observed on the high seas (Gulf Stream) (Bergh *et al.*, 1989; Bratbak *et al.*, 1990).

Bacteriophages range in size from ten to several hundred nanometers. They are composed of a complex protein structure which varies considerably among them. But all phages have a capside which encloses the genetic material. The capside is an assemblage of protein substructures called capsomeres which together form a complex geometric structure (usually an icosahedron or a filament). This structure is sometimes also covered with a lipid coating. Some phages possess a tail (long or short, sometimes even retractable). Various fibres and spikes complete the structure in most cases (Ackerman and DuBow, 1987).

Bacteriophages are certainly the organisms best suited to hydrology from all available biological tracers. Their size range is similar to viruses of Eucaryotes. In contrast, as shown above, the bacteriophages used in this study are not pathogenic or toxic for humans, animals or plants. Furthermore, a careful and appropriate choice of a phage/bacterial-host system will present no risk to the aquifer microflora. The phages, even when used as tracers at high concentrations, are not visible. Therefore, ground and surface water flow paths can be tested and analyzed even in inhabited urban and rural areas. Another benefit of using phages as tracers comes from the specific affinity of a particular bacteriophage to its unique bacterial host. Because of this biological phenomenon, several different bacteriophages can be injected at the same time in the same aquifer. Their enumeration is done without any interference between the phages as different bacterial hosts

Table 1. Bacteriophage habitats [1]

Water	Surface water	Lakes, ponds, rivers, tanks, hot springs, salt marshes, lagoons
	Sea water	Open ocean, coastal and deep marine sediments
	Wastewater	Treated sludge
Soil	Types	agricultural, clay, compost, forest, swamps, podsol, sand
Air		aerosol particles, dust particles
Plants	Organs	shoots/buds, leaves, nodular legumes, root vegetables, grains
Animals	Species	Man, bees, goats, hens, crabs, earth worms, squirrels, horses, various fish, mussels
Food	Milk Products	Milk, cream, butter, milk cheeses (Cheddar, cottage), fermented cheeses (Bel Paese, Emmenthal), yogurt
	Meats	Chicken, beef, sausages
	Other	Oysters, wine, fermentation products for sake

[1] Data modified from Keswick *et al.*, 1982.

are used for the analysis (see Rossi & Käss, 1998, for more details on the analytical method).

One litre of laboratory culture contains from 10^{12} to 10^{14} bacteriophages. This quantity of organic material, weighing about 100g, is small and presents no possible risk of organic pollution. The detection methods of the analyses have a sensitivity of the order of 1 to 2 phages per ml under routine analysis conditions. This is at least equal to, and in many cases better than, the best chemical tracer or spectrofluorometric analysis.

Phages are stable over periods of from several weeks to months, depending on the phage and the conditions *in situ*. This means phages will be effective during tracer tests in most surface water and karst environments, in discrete or well connected fracture studies and in moderate to high permeability porous media settings. In nature, injected phage concentrations degrade exponentially in aquifer or surface water effectively leaving no residual background levels. Detailed laboratory studies have shown that the degradation (or inactivation) rates of phages are influenced by diverse physical and chemical factors (temperature and ionic charge) and by the presence of colloidal particles, minerals and organic matter (clays and humic acids) (Rossi, 1994).

Results

Results of tracer tests conducted in Swiss surface water, karst and porous media environments have been selected to illustrate the comparative behaviour of phages and conventional tracers.

SURFACE WATER – AREUSE RIVER

A comprehensive and detailed sampling and measurement programme took place along almost 28 km of the Areuse river system in Canton Neuchâtel in September, 1994. Details of the 12 sampling and measuring stations are in Table 2.

Injection conditions

Injection Point: St. Sulpice (NE), 750 m from the Spring
Date: Sep. 20, 1994 **Time:** 11:45 hr.
Co-ordinates: 532.980/195.880 **Altitude:** 755 m
Flow rate at injection: 4.9 m^3 sec^{-1}
Duration of injection: 20 seconds
Volume of flow influenced: 98 m^3
No. of sampling points: 12 locations
Uranine: 4.5 kg, diluted initially in 30 L of water
Bacteriophage-Type-H40/1; Quantity: 22.5 L of culture containing about 2.9×10^{15} phages

The first three points (Fleurier, Aval Fleurier, Boveresse) were sampled manually. All other locations were equipped with either ISCO or Buehler automatic sampling devices. Measurements were made or samples taken every 15 minutes and every three samples were made into a single composite for analysis. Flow rates in the river were measured at five different points. The tracer test was conducted during a relatively stable period of flow in September, 1994. The river flow decreased by about 10 per cent at Cortaillod during the 14 hour measurement period ($11.3m^3$ sec^{-1} at 10:00 hr on Sep. 20, 1994 to $9.8m^3$ sec^{-1} at 24:00 hr).

Table 2. Areuse River sampling station data.[1]

Station	Swiss N & E Coordinates	Altitude (m)	Distance (km)	Slope (%)	Avg. flow rate (m^3/sec)
1. Fleurier	533.950/195.380	744	1.25	0.88	4.9 [2]
2. Aval Fleurier	535.470/195.400	735	2.85	0.56	5.5 [2]
3. Boveresse	537.150/196.820	732	5.25	0.13	NM [3]
4. La Presta	540.060/198.160	728	8.55	0.12	NM
5. Sur-le-Vau	543.400/199.680	725	12.65	0.07	NM
6. Noiraigue	545.750/200.370	722	16.75	0.007	NM
7. Moyats amont	548.080/200.400	620	18.85	4.86	9.8 [4]
8. Moyats aval	548.365/200.470	620	19.15	4.25	9.8 [4]
9. Combe Garot upstream	551.070/201.510	529	22.80	2.49	NM
10. Combe Garot downstream	551.200/201.470	529	22.95	2.39	NM
11. Chanet	553.620/201.000	455	25.75	2.64	NM
12. Cortaillod	555.430/200.300	439	28.75	0.53	9.8 [4]

[1] Work done by Gretillat (Bureau Matthey, Montezillon, NE) with Hydrogeology Centre and Microbiology Laboratory of the University of Neuchâtel.
[2] Flow measurements made in place with meters
[3] NM: not measured
[4] Flow measurements from calibrated stream level gauge.

1st Reach—From St. Sulpice to Noiraigue

Breakthrough curves and related data for the uranine and bacteriophages given in Figs. 1 and 2 and Table 3. The first reach of the river is characterized by a relatively low slope. Low flow rates occurred during the test and were further reduced by the low water levels and the bedrock sills that in many parts of the reach extend laterally across the river bed.

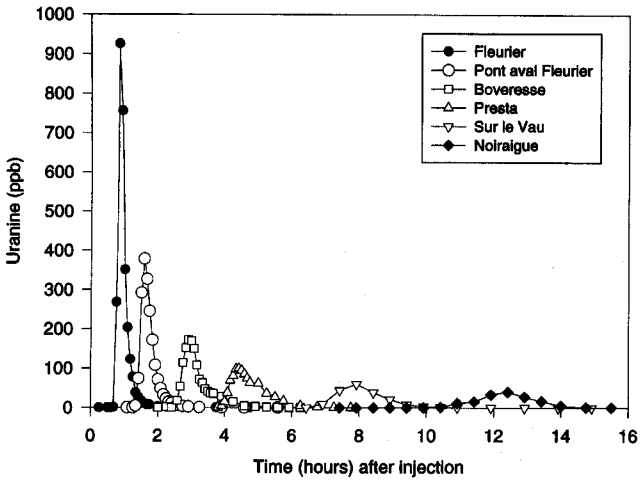

Fig. 1 *Uranine breakthrough curves along the 1st reach of the Areuse River between St. Sulpice and Noiraigue.*

Uranine concentrations at all sampling points exhibited a classic pattern. Maximum concentration values (C_{MAX}) dropped as a function of the flow rate and the distance from the injection point as the result of dilution. At the same time, the tracer underwent longitudinal dispersion as shown on the breakthrough curves.

Comparison of the uranine and bacteriophage breakthrough curves is straightforward. Both have identical distribution patterns. Their dispersion as well as their maximum concentration values are quite similar. The data

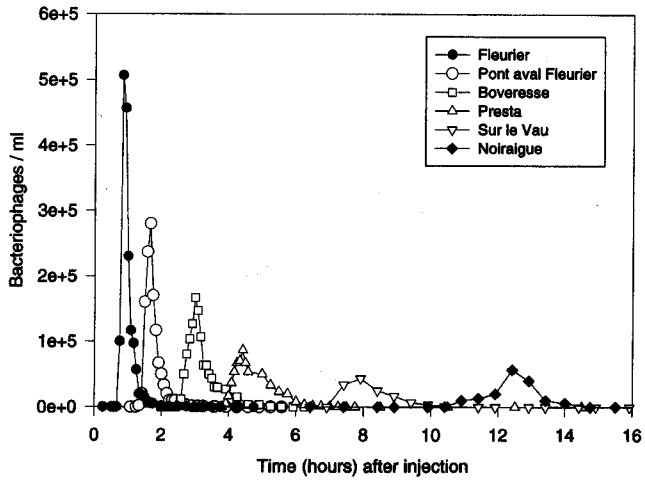

Fig. 2 *Bacteriophage H40/1 breakthrough curves along the 1st reach of the Areuse River between St. Sulpice and Noiraigue.*

suggest, therefore, that the bacteriophages and uranine migrated in a similar manner. The only noticeable behavioural difference between the two tracers was the first arrival time of detectable amount of tracer (T_{ARR}). In general, the phages appeared to arrive earlier than conventional tracers, even when their maximum observed or peak concentrations (T_{MAX}) occurred at the same time. This phenomenon of earlier phage arrival also occurred in a similar manner during tests in fractured karst media (see below). In fact it is believed that the higher sensitivity of bacteriophage analysis accounts for this apparent earlier arrival. The phage analysis detection limit of 1 phage per 2 ml is much lower than the uranine detection limit of the order of 5×10^{-10} g ml^{-1} (0.5 ppb), which represents about 8×10^{11} molecule ml^{-1}. As both tracers probably travel with similar speed, the fluorescent tracer is likely to be present in the first samples where the phages were detected. However, the fluorimeter was not sensitive enough to detect these low concentrations.

Table 3. Areuse River tracer test results for the 1st reach from St. Sulpice to Noiraigue

Station	Uranine				Bacteriophages			
	T_{ARR}	T_{MAX}	V_{ARR} (km/h)	V_{MAX} (km/h)	T_{ARR}	T_{MAX}	V_{ARR} (km/h)	V_{MAX} (km/h)
1. Fleurier	0h40	0h50	1.87	1.51	0h30	0h50	2.5	1.51
2. Aval Fleurier	1h20	1h35	2.14	1.80	1h10	1h25	2.44	1.80
3. Boveresse	2h35	2h55	2.03	1.80	2h25	2h55	2.17	1.80
4. Presta	3h55	4h25	2.18	1.93	<3h55	4h25	>2.18	1.93
5. Sur-le-Vau	6h40	7h30	1.90	1.69	6h30	7h30	1.95	1.69
6. Noiraigue	10h30	11h30	1.60	1.46	8h55	11h30	1.88	1.46

T_{ARR}: time of the first positive sample
T_{MAX}: time of the maximum tracer concentration
V_{ARR}: tracer velocity calculated from T_{ARR}
V_{MAX}: tracer velocity calculated from T_{MAX}

Table 4. Areuse River tracer test results for the 2nd reach from Noiraigue to Cortaillod

Station	Uranine				Bacteriophages			
	T_{ARR}	T_{MAX}	V_{ARR} (km/h)	V_{MAX} (km/h)	T_{ARR}	T_{MAX}	V_{ARR} (km/h)	V_{MAX} (km/h)
7. Moyats upstream	12h00	13h30	1.57	1.40	11h50	13h30	1.59	1.57
8. Moyats downstream	11h30	13h30	1.67	1.42	10h35	13h30	1.80	1.67
9. Combe Garot upstream	14h00	16h00	1.63	1.43	12h35	15h30	1.81	1.47
10. Combe Garot downstream	11h00	13h00	2.09	1.77	<10h35	13h00	>2.16	1.77
12. Cortaillod	13h15	15h15	2.17	1.89	13h00	16h00	2.21	1.80

2nd Reach—From Noiraigue to Cortaillod

The hydrological setting in the second reach of the river is more complex. In addition to having a steeper slope, four hydroelectric facilities disrupt the natural flow along this stretch. The breakthrough curves are not presented here, but a summary of the major results is given in Table 4. In contrast to the results of the 1st reach to Noiraigue, the bacteriophage pattern on the 2nd reach downstream from Noiraigue differs from that of uranine. The chemical tracer seems to have been influenced only slightly by the power stations. The breakthrough curves show a single uranine tracer peak. The bacteriophage breakthrough curves show more peaks, potentially resulting from the multiple water intakes and outfalls from the power stations. Therefore, the phages appear more sensitive than uranine to changes in the surface water hydraulic conditions.

RECOVERY RATE

The quantity of uranine and of bacteriophages recovered at three points on the Areuse were calculated from the automatic and manual flow measurement data (Table 5). According to Gretillat (1994), uranine breakdown in rivers can result from sunlight causing molecular breakdown. A similar behaviour was also observed in tracer experiments on the Aar River (Naturaqua report, 1994).

Recovery of only about 60 per cent of the bacteriophage originally injected can be accounted for by: 1) viral particle inactivation occurring under the varying physical conditions, and 2) adsorption on river materials. Turbulence arising from the flow in the gorges and at the intake and outfall of the power station turbines probably accounted for large losses. Nevertheless, the recovered mass of the phages and uranine at Cortaillod was of the order of about 60 and 50 per cent, respectively. This suggests a total mass recovery 20 per cent greater for phages than for the fluorescent dye tracer uranine.

Figure 3 demonstrates that the maximum concentration values (C_{MAX}) recorded at each measurement point were similar in form for both tracers. The C_{MAX} values change in response to dilution and longitudinal dispersion displayed by the tracers. The breakdown of uranine by sunlight appears to be of the same order of magnitude as the inactivation and adsorption of the bacteriophages.

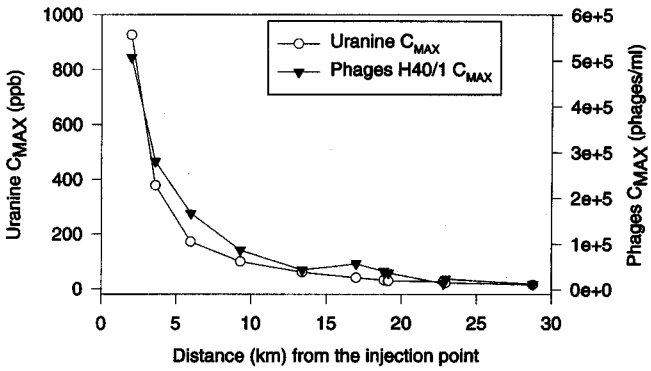

Fig. 3 *Maximum uranine and bacteriophage H40/1 concentrations versus distance to the surface water sampling station from the injection point.*

Table 5. Areuse River tracer test recovery rates.

Station	Uranine		Bacteriophages	
	Mass Recovered	% Mass Injected	Quantity Recovered	% Initial Quantity
1. Fleurier	4.1 kg	91%	$2.44 * 10^{15}$	84%
7 & 8. Les Moyats	3.0 kg	67 %	$2.725 * 10^{15}$	94%
12. Cortaillod	2.2 kg	49%	$1.77 * 10^{15}$	61%

KARST AQUIFERS—AREUSE SPRING CATCHMENT

Over 20 bacteriophage tracer tests have been conducted in the Karst terrain of the Swiss Jura and Alps, some in conjunction with fluorescent tracers tests to compare bacteriophage and conventional dye migration behaviour. The detailed testing in the Areuse spring catchment area in both 1993 and 1995 is presented in this section as an example of this karst environment evaluation.

Tracers were injected into the sinkhole of the Moulin du Lac des Taillères, Switzerland. This sinkhole is in the karst spring catchment basin and is connected to the spring of the Areuse River (Canton Neuchâtel). This river connection has been documented for almost 100 years. The Areuse spring is the principal outlet of this karst aquifer which occurs in the limestone and dolomite of the Upper Malm Formation, which has an average thickness of about 300m. The spring catchment is about 120 km^2 in extent; it drains two relatively large synclinal basins, La Brevine and the eastern part of the Verrieres (see Dörfliger, 1996, for more details).

The spring is characterized by rapid variations in flow rate following rainfall, an equally rapid rise and decline in response to the rain followed by a long period of sustained base flow. This behaviour is typical of karst aquifers and demonstrates the dual response characteristics of flow regimes which arise from the internal structure of karst aquifers. This dual structure stems from 1) the connection of channels, pathways and large cavities forming an underground reservoir with one or more karst outlets, and 2) extensive fissures in the large blocks of otherwise low permeability limestone which eventually contribute to the system base flow.

On two separate occasions, 10 litres of bacteriophages H6/1 and H40/1 were injected simultaneously with a conventional fluorescent tracer; In November, 1993, sulphurhodamine G Extra was the fluorescent tracer used while in February, 1995 it was uranine. The 1993 test was carried out under base flow conditions with a measured average flow rate of 1.25 m^3 sec^{-1}. The 1995 test began under relatively similar low flow conditions but discharge increased suddenly as a result of snow melt so that the average flow rate was 11 m^3 sec^{-1}. Results are given in Table 6 and Figs. 4 and 5.

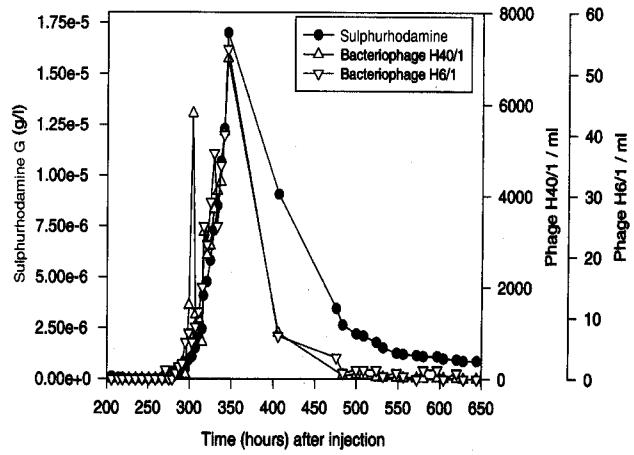

Fig. 4 *Bacteriophage and sulphurhodamine G extra breakthrough curves for the November, 1993 injection at the Areuse Spring.*

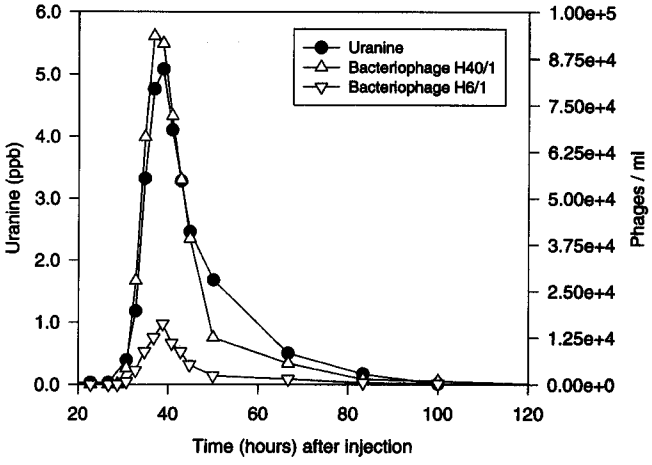

Fig. 5 *Bacteriophage and uranine breakthrough curves for the February, 1995 injection at the Areuse Spring.*

Table 6. Areuse Spring tracer tests – characteristics and results.

Quantity	Tracer	T_{ARR} (h)	V_{ARR} (m/h)	T_{MAX} (h)	V_{MAX} (m/h)	Recovery rate
Tracer Test 1: 3 November, 1993 (distance of 6.25 km)						
8550 g	Sulphur. G.	283	22.1	344.5	18.1	80%
1.49 10^{15}	H6/1	271	23.1	344.5	18.1	1%
9.18 10^{15}	H40/1	243	25.7	344.5	18.1	21%
Tracer Test 2: 21 February, 1995 (distance of 6.55 km)						
5000g	Uranine	31	211.4	39	168	56%
5.71 10^{13}	H6/1	31	211.4	39	168	25%
1.68 10^{14}	H40/1	29	226	37	177	51%

In the 1993 test, the breakthrough curves for bacteriophages and the sulforhodamine G Extra had remarkable similarities (Fig. 4). The T_{MAX} values were similar for both tracers at about 14.3 days with a transit time for the tracers of about 21 days through the system. Phage H40/1 had a higher recovery rate than Phage H6/1 and a concentration peak of 7030 bacteriophages per ml after about 12 days of underground travel. The relatively low, almost negligible, recovery of the phage H6/1 (1%) and the high variability in concentrations could be the result of adsorption reactions onto mineral surfaces such as clay colloidal particles (Rossi, 1994). This adsorption behaviour does not appear to affect sulphurhodamine.

The phage and uranine breakthrough curves (Fig. 5) are similar in the 1995 test. The phage breakthrough curves do not exhibit the same degree of variability that occurred in 1993. In 1995, the transit time of the bulk of the tracer through the system was about 15 times faster than during the low flow period with most of the tracer evident for no more than about two days (from 32 to 80 hours after injection). The C_{MAX} values of the two phages were equally elevated in this test although H40/1 had a more distinct breakthrough than H6/1. The recovery rate of H40/1 was comparable to uranine. For both tests, as was the case for the surface water example, the shorter time for first arrival of the biological compared to the fluorescent tracers is likely the result of the instrumentation sensitivity/analysis detection limit.

GRANULAR POROUS MEDIA AQUIFER—WILERWALD TEST SITE

Three tracer tests were conducted at a ground water research site located on the Emme River fluvioglacial alluvial plain in the Wiler Utzendorf region of Canton Bern. This existing experimental research area had been used to study porous media using geophysical methods (see Carvalho Dill and Müller, 1992, and Carvalho Dill, 1993, for detailed maps of the test field) and also had well documented ground water levels and flow conditions. There are two, 2.5-inch diameter wells suitable for tracer injection and two principal arrays of 20 piezometers screened to depths of about 2, 12 and 14m at distances of about 10 to 15 and 65 to 70m from the primary injection well. The site is of particular interest as it consists of the type of granular aquifers from which increasingly larger volumes of ground water are being pumped for both agricultural and domestic potable water supplies in Switzerland.

The fluvioglacial gravel plain has a sedimentary thickness that at the site has been mapped principally using electromagnetic geophysical methods. Unfortunately, the only two borings that were logged have only limited lithologic data for this relatively heterogeneous subsurface. The slotted metal piezometers were driven into the ground so that the well hydraulics of the sampling conditions and subsurface geologic conditions have to be extrapolated from indirect methods such as geophysics and tracer test data analysis. The water table level was about 1.5 to 3.5m below ground surface during the testing and had a relatively steep gradient of about 0.004 (0.4 per cent) towards the northwest. The aquifer stratigraphy interpreted from the geophysical mapping suggested the presence of several high permeability gravel channels with adjacent silt/finer sand 'islands'. The ancient glacial river bed sand and gravel deposits overlie lower permeability lacustrine deposits at a depth of about 15m. The setting is typical of the glacial stream depositional environments found throughout much of Switzerland's inter-mountain valleys (Rossi and others, 1994).

Tracers were injected using the method proposed by Käss (1992). The water level was initially lowered using a small electric pump placed at a depth of about 8m. The water taken from the well was mixed with the different tracers in plastic containers at the surface. The mixture was then injected at the well head with a siphon from the plastic mixing container. In this manner, a slightly elevated piezometric head was created and the tracer was assumed to have been injected over the width of the screened section. Sampling from the piezometers was done over the entire column of water using tubing repeatedly lowered manually into the wells. The test began on June 6, 1995 following a sustained period of rainfall which had caused relatively high water level conditions at the site.

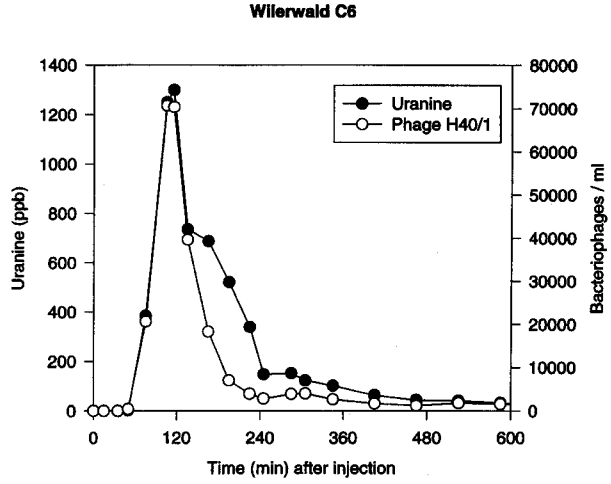

Fig. 6 *Uranine and bacteriophage breakthrough curves in the C gallery well C6 at the Wilerwald site (about 12m from the injection point).*

Injection Conditions

Injection: all tracers were injected simultaneously in well B4

Duration: 5 minutes of pumping during which time the tracers were injected

Table 7. Wilerwald porous media tracer test results for wells C6 (12m) and D7 (64m).

Well	Uranine				Bacteriophage H40/1			
	T_{ARR}	T_{MAX}	C_{MAX}	V_{MAX}	T_{ARR}	T_{MAX}	C_{MAX}	V_{MAX}
C6	75 min	125 min	1300 ppb	6.7 m/h	50 min	105 min	71'000 phages/ml	8 m/h
D7	385 min	790 min	88 ppb	4.9 m/h	325 min	550 min	980 phages/ml	7 m/h

Tracers: 300 g Uranine in 10 L of water;
Phage T7: 1.5 L in suspension containing 10^{13} phages
Phage H40/1: 2 L in suspension containing 10^{13} phages.

Results from monitoring wells C6 and D7 (Fig. 7, Table 7) located at distances of about 12m and 64m from the injection point, respectively, are representative of the migration of the tracers in the permeable channel. The geophysical mapping results (Carvalho Dill and Müller, 1992) indicate that both these piezometers lie in the center of a relatively high ($\sim 10^{-3}$ m s^{-1}) permeability glacial paleochannel.

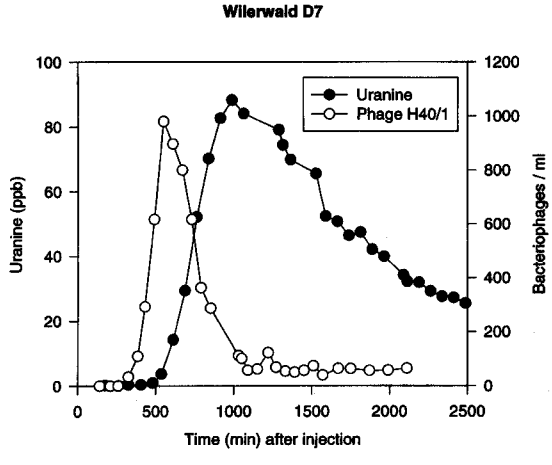

Fig. 7 *Uranine and bacteriophage breakthrough curves in the D gallery well D7 at the Wilerwald site (about 64m from the injection point).*

The bacteriophage breakthrough show that there was a surprisingly fast migration rate of about 8 m h^{-1} to C6, the nearest well and even over longer distances at about 7 m h^{-1} to well D7. Phage H40/1 C_{MAX} values decreased by a factor of about 70 in this 60 m distance from 71000 phages ml^{-1} at C6 to 980 phages ml^{-1} at D7. The results from this and the other earlier tracer tests conducted at the site (not presented in this article) indicate that bacteriophage migration is faster and of higher concentrations in the paleochannels than in other porous material. These channels represent preferential pathways towards the other wells that are more effective for transport than the lower permeability silt, clayey and fine sand 'island' areas.

Clearly, the bacteriophages appear to have been filtered out to some extent in the granular media. In the lower permeability materials, interaction of this particular biological tracer with the silt and clay size particles appears likely to have resulted in phage adsorption from the water onto the mineral particles. Therefore, transported bacteriophages through these types of lower permeability zones should have had a much smaller likelihood of migration. Surprisingly, however, some bacteriophages were always present in the media, even in the lower permeability silty zones. Their migration was observed over 160m in such zones (Rossi and others, 1994) even if their levels were only several phages per ml. However, their rate of migration could be greater than or equal to typical 'conservative' tracers even in such conditions.

Travel times for the phages to reach (T_{ARR}) and peak (T_{MAX}) at both wells were significantly faster than for uranine (Table 7) even if the percentage differences in arrival times decrease with distance away from the injection well. Uranine arrived at well C6 about 25 minutes later than the phages (50 per cent slower) and at well D7 60 minutes later (20 per cent slower). In this instance, based on the values observed for the initial part of the entire testing, it is unlikely that the analysis/detection limit difference for the two tracers can account for such a large discrepancy in arrival times. In contrast, percentage differences in the peak concentration times (T_{MAX}) increased dramatically between the two types of tracers as the distance away from the injection site increased (Figs. 6, 7).

The breakthrough curves also show that, as expected, the dissolved constituent uranine has a larger degree of dispersion than the phage particles. For uranine, the 88ppb C_{MAX} value recorded at D7 was about 15 times less than the 1300 ppb C_{MAX} value for well C6. The phage C_{MAX} values decreased about 70 times between these two wells, indicating that phages are attenuated by *in situ* processes to an extent that is more than four times greater than for the uranine reduction. However, the large phage quantity that is able to be injected in a small volume of liquid at the outset of the tracer test compensates for this. Accordingly, the results from the Wilerwald porous media research site demonstrate that phages represent an effective and quantitatively useful tool for ground water pathway analysis.

Conclusions

The use of bacteriophages as biological tracers in both surface and ground waters responds to the increasing need for investigation methods that have no or reduced risk to the environment. These techniques are not only necessary to develop a better understanding of how natural systems function, but they also provide an opportunity to predict the impact of human activities on their surroundings. The tracing technique developed in a completely multidisciplinary manner corresponds directly to the actual needs of both hydrologists and hydrogeologists. The objective of the work was not to replace conventional fluorescent tracers but to increase the number of tracers applicable to a wide variety of hydrological studies.

For surface water hydrology, the 28km of superficial flow of the Areuse river represents a first in a comparative test between two totally different, dissolved and particulate tracers. The river flow was sampled systematically; based on the extensive density of stations and frequency of sampling, the behaviour of the two tracers has been documented and compared quantitatively. Moreover, this testing allowed determination of the behaviour of microscopic colloidal size particles in a complex flow system consisting of torrential, turbulent as well as laminar flow conditions. The two principal segments studied had dramatically different flow characteristics. The first was characterized by low flow rates of the order of 5 to 8 $m^3 s^{-1}$. In this part of the flow system, the two tracers behaved in an almost identical manner with negligible difference in their arrival times. The second segment had greater relief frequently interrupted and with a much higher slope. In addition, power plants with their intakes and outfalls changed the natural flow conditions radically. Bacteriophage behaviour was different from that of uranine in that a larger number of elevated peaks occurred in their breakthrough curves. This difference may be explained by both dilution and mechanical inactivation caused by the turbines. The second reach in the Gorge of the Areuse demonstrated that phages have a much higher sensitivity to changes in hydrodynamic conditions than classical fluorescent tracers.

In karst terrain, a quantity of 3×10^{15} bacteriophages was injected at the Areuse basin sinkhole in 22L of suspension which mixed quickly with the karst aquifer waters. This quantity was large enough to evaluate a regime with a flow rate of $50m^3 s^{-1}$ based simply on the value of C_{MAX} obtained from the test. Niemela and Kinnunen (1968) have also carried out field tests to show that bacteriophages are effective in tracing flows as high as $262m^3 s^{-1}$ over a reach of 17 km using a volume 20 times smaller than in this study. The method demonstrated here appears to be applicable for use in most karst catchment basins of interest. Moreover, this type of tracer may be used even in situations where there are large quantities of suspended materials in the flow as is generally the case for high slope and velocity stream and cave channel environments. In contrast to uranine analysis the bacteriophage analysis method is not affected by turbidity.

In karst media, bacteriophages have similar results to conventional tracers. Some differences, for example small peaks on the breakthrough curves, may occur particularly under low flow conditions. These peaks may be the result of remobilization of clay particles in the aquifers. Extended laboratory work has shown that the bacteriophages interact more strongly with mineral particles than conventional tracers. Bacteriophages can be adsorbed on fine particles in suspension without any hindrance to their virulence and can be transported over relatively long distances.

In a porous media aquifer, bacteriophage migration was observed to be faster than both chemical and fluorescent tracers. This finding is consistent with trials using napthionate (Rossi, 1994). Now, at this site, phages have been shown to flow faster than other dissolved tracer substances such as uranine and potassium iodide [KI] (Rossi, unpublished). In permeable interstitial porosity aquifers (gravel and sand), bacteriophages have moved over great distances and in large quantities. In the less permeable silty zones, adsorption of bacteriophages on the matrix substrate is believe to be the main cause for the dramatic reduction in the number of particles in suspension. However, at Wilerwald, a small number of phages has travelled through some of the interstitial pore pathways, probably those with the highest migration velocities. The migration velocity of bacteriophage particles corresponds logically to the most rapid flow paths in the granular system. If so, it could be a highly representative method of predicting and characterizing certain types of contaminant flow and behaviour perhaps not as well predicted by conventional tracers. Therefore, this approach needs further development, documentation and testing.

The three hydrological settings for tracer tests presented here demonstrate that bacteriophages are equally applicable to surface water, karst terrain and porous media aquifer investigations. The fact that bacteriophages offer substantial benefits to hydrologists and hydrogeologists in comparison to conventional tracers is an additional incentive to pursue their use in a number of different environments. Bacteriophages, because of their size and structure, are similar to mammalian pathogenic viruses and to a lesser extent, to bacteria. The use of a selected phage type may simulate the behaviour of pathogenic viruses as well as certain other types of bacteria in waters more accurately. Porous media aquifer protection zones have typically been defined using conventional tracers. Yet, the technique using bacteriophages could prove more applicable and should now be considered strongly for use in these cases. This method of tracer testing using bacteriophages is also pertinent in the light of increasing concerns of environmental protection. Results from the last few years of applying this method have shown that it is a precise and modern tool applicable not only to qualitative

reconnaissance studies, but to detailed quantitative analysis in diverse hydrological conditions.

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